Video Article

Maintenance of a *Drosophila melanogaster* Population Cage

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URL: https://www.jove.com/video/53756
DOI: doi:10.3791/53756

Keywords: Molecular Biology, Issue 109, Fly, population, cage, Drosophila, embryo, larvae, pupae

Date Published: 3/15/2016

Citation: Caravaca, J.M., Lei, E.P. Maintenance of a *Drosophila melanogaster* Population Cage. *J. Vis. Exp.* (109), e53756, doi:10.3791/53756 (2016).

Abstract

Large quantities of DNA, RNA, proteins and other cellular components are often required for biochemistry and molecular biology experiments. The short life cycle of *Drosophila* enables collection of large quantities of material from embryos, larvae, pupae and adult flies, in a synchronized way, at a low economic cost. A major strategy for propagating large numbers of flies is the use of a fly population cage. This useful and common tool in the *Drososphila* community is an efficient way to regularly produce milligrams to tens of grams of embryos, depending on uniformity of developmental stage desired. While a population cage can be time consuming to set up, maintaining a cage over months takes much less time and enables rapid collection of biological material in a short period. This paper describes a detailed and flexible protocol for the maintenance of a *Drosophila melanogaster* population cage, starting with 1.5 g of harvested material from the previous cycle.

Video Link

The video component of this article can be found at https://www.jove.com/video/53756/

Introduction

The ability to combine genetic and biochemical approaches has made *Drosophila* a particularly suitable organism for biochemistry and molecular biology studies[1-3]. These studies often require large amounts of biological material, not only from adult flies, but also from larvae[4], pupae[5] and embryos[6-8]. To obtain large quantities of material, researchers have cultured flies using large containers known as fly "population cages". These cages consist of a cylinder made of plastic covered by a net on both sides to allow the introduction of the food inside the cage without the flies escaping. These cages can be homemade[9-11] or purchased from a company (see table of specific materials/equipment).

A major advantage of using this system to grow large numbers of flies is that the cycle of the fruit fly[12] can be controlled in a way that all the flies develop in a relatively synchronized manner. This synchronization is achieved by seeding new embryos, feeding larvae/flies and sacrificing the adult flies at precise times. Using a synchronized fly population is particularly useful for developmental studies[13].

The start of a new population cage from a few flies is a time-consuming process requiring many cycles of amplification [9-11]. Even using bigger containers like fly culture bottles or minicages, the whole process can last for months. To avoid this time consuming step, many *Drosophila* laboratories regularly maintain such cages. It is most convenient to start a new cage starting from an embryo collection from an already established population cage. In general, most labs maintain wildtype population cages, such as Oregon R or Canton S. This manuscript presents a detailed protocol to maintain fly population cages.

Protocol

1. Starting the Cycle: Seeding Embryos

NOTE: The cycle starts with 1.5 g of collected material (a mixture composed of embryos and/or some first instar larvae) from the previous cycle. This material will be placed in a plastic container (see table of specific materials/equipment) with an active yeast mixture until the pupal phase. The container will be closed after the introduction of the biological mixture of embryos and larvae with the lid to avoid allowing the larvae to escape. It is necessary to make holes into the lid in order to allow air circulation. To avoid having larvae escape through the holes, foam plugs are used. Finally, It is recommended to wear gloves and lab coat, not only in this section but also in the whole protocol, to avoid getting dirty or staining clothes with bleach.

1. Set up the plastic container.
    1. Make three square holes with a razor blade in the plastic container lid of approximately 2.2 cm. Add tape (3/4 inch labeling tape) to the four corners of each hole to ensure a tight fit for the foam plugs (50 x 55 mm, d x l), and then place one foam plug in each hole.

2. Cover the inside of the plastic container with plastic wrap. Over this film, add a layer of cotton to cover the bottom of the plastic container. Tear the cotton with your fingers.

2. Prepare the fly food.
    1. Add 333 ml of deionized water in a 500 ml beaker. While stirring the beaker with a magnetic bar, add 167 µl of propionic acid and 1.08 ml of phosphoric acid, from the stock solutions (99.96% and 85%, respectively).
    2. Slowly add 77.5 g of active dry yeast, avoiding big clumps. After dissolving the dry yeast, add 38.8 g of sucrose.
    NOTE: Sucrose should be the last ingredient to be added because immediately after adding this component, fermentation will begin.

3. Immediately after dissolving the sucrose, pour the food over the cotton, and make sure to cover the cotton evenly. Close the plastic container with the lid to avoid escaped flies within the laboratory contaminating the food.
4. Resuspend 1.5 g of the harvested embryos (not dechorionated) from the previous cycle with 5 ml of 70% ethanol. Cut in half two filter papers and distribute the biological mixture evenly over the 4 pieces using a spatula or a transfer pipet with a wide tip (cut if necessary). Lay the filter papers on top of the soaked cotton and close the lid. Finally, incubate the plastic container at RT (24ºC) and humidity (35%) until the pupa stage.
    NOTE: The plastic container should not be placed in a humid chamber, otherwise this will encourage the growth of bacteria.

2. Continuation of the Cycle: From Embryos to Flies.

NOTE: This part of the cycle goes from the embryos placed in the plastic container on day 1 until 9 days later when the adult flies will emerge from the pupae. During these 8 days nothing has to be done but monitoring that the embryos progress correctly to the next stages of the *Drosophila* life cycle until eclosion. If larvae start dying and turning black during this time, check that the foam plugs are not too tight and that adequate ventilation is provided. This period is a good time to clean the fly population cage from the previous cycle.

1. Observe the embryos become 1st instar larvae around 24 hr after the adult female deposited them into the fly food (see step 3). The resulting larvae will feed from the fly food prepared in step 1 during 4 days, growing and molting twice into 2nd and 3rd instar larvae.
2. Clean the fly population cage.
    1. Place the cage at 4 ºC for at least 30 min to slow down the activity of the flies. Remove the net that is covering one side of the cage, and with a fast movement, cap the open side with a large biohazard plastic bag. Pour the contents of the cage into the bag.
    2. With the biohazard bag still covering one side of the cage, position the cage vertically over a sink with the biohazard bag side down. Carefully remove the plastic bag to prevent flies escaping and discard it in a biohazard waste container.
    3. Carefully open the upper net enough to create a small hole, rinse the inside of the cage with water, which will wash the flies into the sink. Slowly increase the size of the hole and eliminate all the flies inside the cage.
    4. Clean the cage and both nets with water and soap. While the nets are still wet secure them on both sides of the dry clean cage, and finally put inside a lab soaker paper, which will prevent the plastic film prepared in step 1.1.2 from sticking to the cage. Now the cage is ready for the next cycle.

3. Four days after the initial setup, observe the larvae pupate. The larvae will remain in this stage for 4 more days.
    NOTE: During this phase the pupae will cover the entire cotton surface inside the plastic container.
4. During the pupal phase and before the first flies emerge, open the plastic container and place the plastic film containing the cotton with all the pupae over the lab soaker paper inside the clean population cage. Close the net with a double knot and clean the plastic container and the lid for the next cycle.
    NOTE: Pupae affixed to the lid should be discarded and destroyed by autoclaving or freezing before eclosion.

3. Adult Flies.

NOTE: After the 4 days of the pupa stage the first flies will emerge from the pupae. Within 24 - 48 hr all flies should have eclosed. In this part of the cycle, it is important to provide them food with the aim of creating the right environment for reproduction.

1. Prepare the molasses trays.
    1. Combine in a 1 L flask 556.25 ml of deionized H_2O, 90 ml of molasses, and 22 g of agar. Autoclave with a stir bar for 30 min, cool and add 9.25 ml of 10% Tegosept in 95% EtOH.
    2. Pour the mixture into a meat tray of 21 x 14.5 cm. Ensure that the volume is enough to cover 15 - 17 trays. Wait 10 - 20 min to solidify and cover each tray with a plastic film to avoid drying. Several flasks of 1 L can be prepared at the same time. Store the molasses trays at 4 ºC wrapped in plastic until required.

2. Add a molasses tray covered with wet yeast inside the cage with the adult flies.
    1. Before adding the plate, place the cage at 4 ºC in the cold room for 30 min.
    2. Add 200 ml of deionized H_2O into a beaker and slowly pour dry yeast while stirring with a spoon. Stop adding dry yeast when the mixture becomes approximately the consistency of peanut butter to prevent flies getting stuck into the food.
    NOTE: It is important not to allow the food solution to solidify otherwise it will be very difficult to cover the molasses tray and extract the eggs during the harvesting process (see section 4.3).
    3. Remove the plastic film from one molasses tray and cover it with a layer of the fresh prepared wet yeast using a spoon or spatula. Be sure that the entire surface is covered.
    4. While the cage is still in the cold room, open the net and carefully add the molasses tray with wet yeast into the cage. Close the net and place the cage at RT.
    NOTE: It may be useful to cover the plate with an empty meat tray during insertion, but be sure not to block access of the flies to the molasses tray.

3. Change the food plate every 24 - 48 hr to avoid the yeast to get too dry. Adult flies are very sensitive to desiccation.

4. End of the Cycle: Harvesting the Embryos

NOTE: The best fly fertility is 3 - 5 days after eclosion. Therefore, harvesting during this time will get the best embryo yield. After desired collections are completed, the cycle is over. The flies should be sacrificed and the cage cleaned as specified in step 2.2.

1. Typically, 2 days after fly eclosion, introduce a new molasses tray with fresh wet food into the cage, as explained in section 3.2.5. To increase the yield of eggs deposition, with the help of the fingers, make the surface of the wet yeast as irregular as possible. If an old food tray is present in the cage, before adding the new plate, carefully remove it and discard it into a biohazard bag. Another tip for increasing the yield is to remove the cotton layer so all the eggs will be laid in the food. Maintain the cage at RT.
2. Observe the embryos as small white dots.
   NOTE: The eggs are ready to be collected. For routine maintenance of the fly cage, 2 day collection is the recommended time. This collection will contain mostly embryos and few 1st instar larvae. See representative results for typical yields of harvested embryos for various collection time points.
3. Harvest the embryos
   1. Place the cage in the cold room for about 30 min. In the mean time prepare the plastic container and the fly food for the next cycle as explained in section 1.
   2. In an 8 L autoclave tray add water to ¼ of the total capacity and then add 1 ml of 10% Triton X-100. While the cage is in the cold room remove carefully the molasses plate and place it inside a biohazard bag.
   3. Remove the tray from the bag and submerge it quickly (to prevent flies from escaping) in the detergent solution in the autoclave tray. Close the biohazard bag and put in a biohazard container. Flies will not drown in water that does not contain detergent.
   4. Use a large paintbrush and distilled water gun to wash embryos and yeast off of molasses. Discard plates and molasses into biohazard box.
   5. Pour solution carefully through a three sieve set (coarsest sieve 30 on top, 40 in the middle and 100 on bottom). Wash clumps on top level with distilled water gun until no clumps remain.
      NOTE: The adult flies and 3rd instar larvae will be retained in the sieve 30.
      1. Rinse the autoclave tray with distilled water and pour the rinse water through the sieves. Remove top sieve and repeat process if yeast clumps remain. Most of the 1st and all of the 2nd instar larvae will remain in the sieve 40.
   6. Remove the second sieve. The yellowish material in the third (bottom) sieve is the mixture of embryos and small 1st instar larvae. Use distilled water gun to move all the eggs to one side of the sieve. Collect them with a spatula and weigh them.
   7. During these cycles the collection yield of embryos/larvae ranges between 7 and 13 g. Use only 1.5 g of this material to start a new cycle (section 1). Use the rest of the embryos for further processing or can be discarded.

5. Further Processing

NOTE: The embryos not utilized for continuation of the population can be used immediately for experiments or alternatively can be frozen at -80 ºC. For both options, the embryos may need to first be dechorionated.

1. For dechorionation, wash the embryos for 2 min in 50% bleach solution and rinse thoroughly with distilled water.
2. To freeze the embryos, first dry by pressing firmly with a paper towel, and then weigh and place them, with the help of a spatula, into a 15 or 50 ml conical tube. Finally submerge the conical tube in liquid N_2 for a few seconds.

Representative Results

The maintenance of a fly cage population is based on the fly life cycle. Therefore, after placing the initial biological mixture of embryos and larvae in the plastic container (**Figure 1A**) the fertilized eggs will become larvae in no more than 2 days and the larvae will grow for 4 days, relatively synchronized through the different instar larvae stages (see **Figure 1B**).

After the larvae have completed the 3rd instar larval stage they will pupate and cover the surface of the cotton inside the plastic container with some on the internal surface of the lid (**Figure 1C**). This pupation period will go for the next 4 days until the flies finish the metamorphosis process. During this period it is strongly recommended to transfer the flies into the cage. The first adult flies will start to eclose at day 9 - 10 after the initiation of the cycle (**Figure 1D**) and by day 11 all of them will have emerged from the pupae.

The best yield of the embryos collection is obtained 3 - 5 days after eclosion (day 13 to 15) and finally declines in day 17 (7 days after eclosion). Therefore, it is recommended to add the last tray of fresh food at day 13 and collect the eggs at day 15 (**Figure 1E** and **F**). This will allow collection of the maximum number of embryos. Adding a third extra day before collecting may result in the food being too dry and consequently will diminish the yield of collected embryos. **Table 1** shows the yield of 6 consecutive cycle collections, with a starting material of 1.5 g in each cycle, and **Figure 2** is the recommended schedule for a whole cycle collection.

The material harvested in 2 day periods is mostly composed of embryos. However a small number of larvae are also present. For cage maintenance purposes these larvae are not a problem, since some degree of asynchronicity is expected. Nevertheless for some biochemistry experiments, the purity of these embryos is very important, but at the same time it is also necessary to collect large quantities of eggs to perform these experiments. For this reason several short consecutive collections were performed at 1 hr and 3 hr, after quickly adding at RT a new plate with food, to provide an estimation of the yield and purity that can be obtained with this method (**Table 2** and **Figure 3**). The 1 hr consecutive collections started with a very low yield (14.5 mg) but then the quantities of embryos increased for each time collected but the last time. The low yield at first may be due to the flies being stressed during the process of changing the food but later acclimating. On the other hand, no larvae were observed in the five sequential collections (**Figure 3A**), showing that shorter collections yield higher purity. If cotton is not removed from the cage, the likelihood that larvae from earlier time points will remain and enter the fresh plate is increased. Even when cotton is removed, at times larvae will wander on the side of the cage and enter the food during a collection.

For the 3 hr consecutive collections, the yield ranged from 840 to 1250 mg, which is around 10 times more than the yields obtained in the 1 hr collections. The purity of these embryos was near 100% (**Figure 3B**). Occasional larvae were observed. Some developmental studies require a very strict synchronization for the embryos collected. To increase the synchronization purity, it is recommended to discard the first collection plate because if conditions are not ideal, females can retain more mature eggs and deposit them when conditions improve, such as with introduction of a fresh plate. Also it is important to know that older adult flies (>6 days) produce less synchronized embryos. To verify the degree of synchronization with high accuracy, a DAPI staining of the collected embryos is recommended.

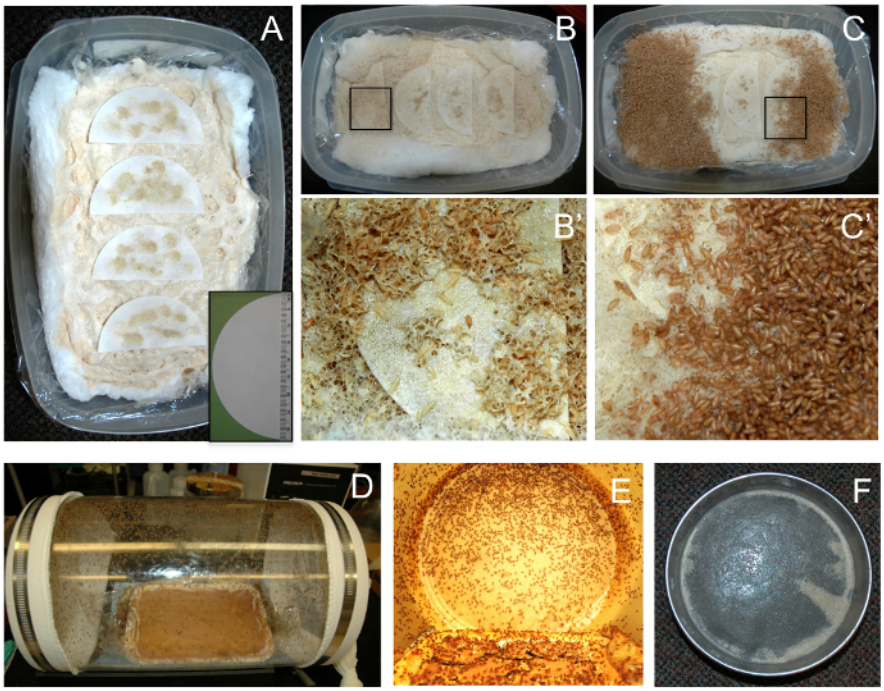

Figure 1. The *Drosophila melanogaster* Life Cycle in a Fly Population Cage. (**A**) Initiation of the cycle seeding 1.5 g of embryos into the plastic box container. As a reference, in the lower panel, the length of the filter paper is 8.5 cm. (**B**) Larvae growing relatively synchronized 5 days after the initiation of the cycle. (**C**) At day 8, most of the flies are in the pupa stage. The lower panels in **B** (**B'**) and **C** (**C'**) are magnifications of the indicated areas in the upper panels. (**D**) Adult flies emerged from the pupae 10 days after cycle initiation. (**E**) Adult flies inside the population cage before starting the process of collecting the embryos, at day 15. The yellowish material on the fly food are fertilized eggs. (**F**) Embryos (and a few larvae) collected at the bottom of the 100 coarsest sieve, after a whole cage cycle. Please click here to view a larger version of this figure.

Sun	Mon	Tue	Wed	Thu	Fri	Sat
			Day 1 Start collection cycle Embryo	Day 2 Emb./Lar.	Day 3 Larvae	Day 4 Larvae
Day 5 Lar./Pup.	Day 6 Pupae	Day 7 Pupae	Day 8 Add pupae to the cage Pupae	Day 9 Pup./Adu.	Day 10 Add first molasses tray Pup./Adu.	Day 11 Adult fly
Day 12 Adult fly	Day 13 Add second molasses tray Adult fly	Day 14 Adult fly	Day 15 Finish collection cycle Adult fly			

Figure 2. Whole Cycle Collection Schedule. Figure 2 shows the recommended schedule for a complete cycle collection. Please click here to view a larger version of this figure.

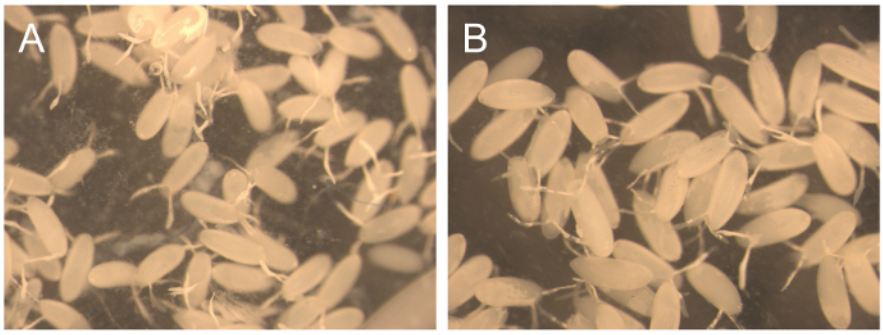

Figure 3. Purity of 1 hr and 3 hr consecutive collections. Representative image of embryos collected at 1 hr (**A**) and 3 hr (**B**) after adding a new tray of food. Please click here to view a larger version of this figure.

Cycle number	Yield (g)
1	10.3
2	9.5
3	10.3
4	12.7
5	10
6	7.1

Table 1. Yield of Several Cycle Collections. Table 1 shows the yield of 6 consecutive cycle collections, with a starting material of 1.5 g. In each cycle, the last tray of food was added at day 13 and the harvest was done at day 15.

Collection #	Length of collection (hr)	Yield (mg)
1	1	14.5
2	1	21.6
3	1	56.1
4	1	160.1
5	1	106.1
1	3	960
2	3	840
3	3	1250

Table 2. Yield of 1 hr and 3 hr Consecutive Collections. **Table 2** shows the yield of 5 consecutive collections 1 hr after adding new food and 3 consecutive collections 3 hr after placing a new molasses plate.

Discussion

Starting with 1.5 g of material one can obtain a yield of collected embryos between 7 and 13 g per cycle. To get such an amount of material it is crucial to maintain the right culturing conditions for all the stages of the fly cycle.

The most important parameters are temperature and humidity, which should be 24 ºC and 35% respectively. If these two parameters cannot be held constant in the normal environment of the lab, one possibility would be to place the fly cage into a incubator or an environmental chamber. Other protocols recommend 70% humidity and also a constant 24 hr light-dark cycle to increase the yield of the produced eggs[9,10]. However keeping the humidity around 35% avoids bacterial contamination, and since the purpose of this protocol is only the maintenance of a population cage, the flies are kept in the normal light environment of the lab.

Another important point is to keep disturbances to the adult flies as low as possible. It may be advisable to keep the cage in a location separate from the fly room to avoid cross contamination from other flies.

The culturing of large populations of transgenic and mutant flies is not recommended, since it is very difficult to maintain their purity and they can exhibit pronounced abnormal mating behavior in large population cages[14].

One possible problem while culturing *Drosophila* in large quantities is the presence of other organisms like mites and/or mold, which will compete for the food and therefore reduce the yield of produced eggs. To avoid this, it is very important to keep all the equipment clean, washing the cage, nets and fly boxes with water and soap, and discarding the disposable material (foam plugs) after every cycle. To reduce mold growing, propionic and phosphoric acid are added into the wet yeast when preparing the fly food in step 1.2 and Tegosept when preparing the molasses tray in step 3.1. It is sometimes helpful to place materials such as the plastic box or sieves in -20 ˚C when time is short and materials cannot be cleaned right away.

A whole collection cycle occurs in 14 - 15 days, beginning when the embryos are seeded into the fly box and ending with the last collection day. During this period of time, it is recommended to organize a schedule in order to remember all the necessary steps for the maintenance of a population fly cage (**Fig. 2**), detailed in the protocol section. From the day that the eggs are seeded, until the adult flies emerge, it is necessary only to inspect the plastic box, and when pupation occurs, to place them inside the cage. After that, the flies have to be fed every 2 - 3 days until embryos are seeded for a new cycle. In the whole protocol, the longest day is during the collection and seeding of the embryos for starting a new cycle. As commented in the protocol, the best egg yield is 3 - 5 days after adult eclosion, and finally declines 2 days later. This gives us some flexibility in order to choose the day in which the harvest of the eggs will be performed.

If for any reason the yield of collected embryos is less than the desired starting amount (1.5 g), one always can add a new molasses tray and collect more eggs the next day. For constant collections, it is recommended to maintain 2 cages in parallel, and if higher quantities of embryos are required, it is also is possible to use bigger cages. In case of doing short time collections, one way to increase the yield is to take advantage of the egg-laying burst in the morning.

There are many advantages of collecting large amounts of various developmental stages. For example, collected embryos from population cages have been used very successfully in immunoprecipitation assays [6-8], mass collection of larval tissues from dissociated larvae has demonstrated to be a very good source for 3C experiments[4] and RNA preparations[15], and heads from adult flies have been utilized for ChIP experiments[16]. In addition, adults are often needed to make fly extracts for tissue culture[17].

One of the most promising applications of the cage is to provide material for high-throughput assays that allow the analysis and screening of genes, transcripts, proteins and metabolites in response to the exposure of pathogens, biological molecules, chemical substances and ionizing radiation. In these large-scale assays large numbers of individuals are required, and the fly population cage described here can be very helpful in order to obtain great quantities of material during the different phases of the *Drosophila* life cycle for their analysis and screening[18].

Disclosures

The authors have nothing to disclose.

Acknowledgements

We thank Yixian Zheng (Carnegie Institution of Washington, Baltimore, MD) for the original protocol and assistance in initial setup and members of the Lei laboratory for critical reading of the manuscript. This work was funded by the Intramural Research Program of the National Institute of Diabetes and Digestive and Kidney Diseases.

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